

108. THE SYNTHESIS OF PHOSPHOLIPIN IN RATS FED ON THE FAT-DEFICIENT DIET

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THE determination of the activity of the lipin-P in rats which have received injections of radioactive sodium phosphate some hours before being killed has been used by various observers to throw light on the synthesis of the phospholipins. It has been established by this means that constant synthesis and degradation of phospholipins is taking place and that this process is much more active in the liver and kidney than in the muscles [Hevesy, 1940]. Similar results were obtained when elaidic acid was used as an indicator [Sinclair & Smith, 1937].

The injection of radioactive phosphate into the fat-starved rats provided a method of comparing their power of synthesizing phospholipin with that of rats which had received, in addition to the fat-free diet, supplements of the essential unsaturated acids. Three groups of the rats, described by Hume *et al.* [1940], were placed at our disposal for this purpose. Group II comprised rats dosed for 38 days with methyl arachidonate following 163 days on the fat-free diet: Group III contained 8 rats kept without fat for 215 days: Group IV included 7 rats which had received supplements of linoleic acid for 60 days before their death. 4 hr. before they were killed each rat was injected with 0.5 ml. of a solution of radioactive phosphate of known activity which could subsequently be compared with a known standard.

The phospholipins extracted from the muscles, kidneys and livers of these three groups of animals were purified by the method of H. MacLean [1914]. The hydrolysate of the phospholipin was taken to dryness, the residue ashed with conc. H_2SO_4 and made up to a known volume.

One-fifth of this was retained for estimation of the phosphorus by Brigg's modification of Bell and Doisy's method. In order to ensure better precipitation when little phospholipin-P was present, 5.5 mg. P were added as sodium phosphate to the remaining four-fifths of the solution and the total phosphate precipitated as magnesium ammonium phosphate. The radioactivities of equal weights of the precipitates derived from phospholipin and added phosphate were determined and from these were calculated the activities of equal weights of the precipitates derived only from phospholipin-P. For convenience of measurement when comparing the precipitates of the liver phospholipin phosphates from Groups IV and II the former was diluted 5.3 times and the latter 12 times. There was not quite sufficient of the active phosphate to give the seventh rat of those receiving linoleate its full dose and a compensating factor of 1.057 has therefore to be introduced into the calculation for this group.

Table 1. *Showing relative activities of phosphate precipitates from phospholipins*

	Liver				Muscle				Kidney			
	mg. Phospholipin-P	mg. Total P	mg. ppt.	Relative* activity	mg. Phospholipin-P	mg. Total P	mg. ppt.	Relative activity	mg. Phospholipin-P	mg. Total P	mg. ppt.	Relative activity
Group II arachidonate	20.06	25.56	212.4	375	7.12	12.62	102.2	96	2.55	8.05	57.8	650
Group III fat-free	28.40	33.9	260.7	455	9.92	15.42	116.6	143	4.37	9.87	67.2	810
Group IV lineolate	26.90	32.4	249.6	916	4.92	10.42	79.4	74	4.08	9.58	77.3	966

* Relative activity = percentage of standard preparation.

$$\frac{\text{Liver}_3}{\text{Liver}_2} = \frac{455}{375} \times \frac{339}{284} \times \frac{2006}{2556} = 1.14$$

$$\frac{\text{Muscle}_3}{\text{Muscle}_2} = \frac{143}{96} \times \frac{15.42}{9.92} \times \frac{7.12}{12.62} = 1.34$$

$$\frac{\text{Kidney}_3}{\text{Kidney}_2} = \frac{810}{650} \times \frac{9.87}{4.37} \times \frac{255}{805} = 0.89$$

$$\frac{\text{Liver}_4}{\text{Liver}_2} = \frac{916}{375} \times \frac{32.4}{26.9} \times \frac{2006}{2556} \times \frac{5.3}{12} \times 1.057 = 1.08$$

$$\frac{\text{Muscle}_4}{\text{Muscle}_2} = \frac{74}{96} \times \frac{10.42}{4.92} \times \frac{712}{1262} \times 1.057 = 0.97$$

$$\frac{\text{Kidney}_4}{\text{Kidney}_2} = \frac{966}{650} \times \frac{958}{408} \times \frac{255}{805} \times 1.057 = 1.16$$

These figures show that the amount of radioactive P taken up in the phospholipins of similar organs of these three groups of rats is surprisingly constant. Only in the case of the muscle tissue of the fat-free rats is there any significant variation. The turnover of P in the muscle phospholipin in the rats receiving only the fat-free diet is about one-third more active than in either of the groups which have received the supplements of unsaturated acids. This result is of particular interest since the evidence from the investigation of the R.Q. of rats with fat-deficiency disease suggested that more fat was being burnt than in the normal rat. Thus Wesson & Burr [1931] showed that in such rats in the first hours following carbohydrate feeding, the R.Q. is well above 1, indicating formation of fat from carbohydrate and that rats in the early stages of the disease show assimilatory and basal metabolic rates 25% greater than normal. This result was confirmed by Burr & Beber [1937] who found a much higher metabolic rate for the fat-starved rats; they state that for 12–16 hr. a day the R.Q. of fat-starved rats remained above 1, but when the rats stopped eating the R.Q. dropped rapidly, indicating the burning of much fat.

Since in the experiments described by Smedley-MacLean & Nunn [1940] the proportions of fatty acids in the liver, kidney and muscle tissues of the rats suffering from fat-deficiency showed no diminution, it could be inferred that the formation of fatty acid from carbohydrate and its conversion into phospholipin proceeded normally. The results of the radioactive injections show that in animals almost devoid of the higher unsaturated acids there is no diminution in the turnover of P in the liver and kidneys and presumably therefore phospholipin is being synthesized as actively in the rat suffering from long-standing fat deficiency as in those with the more normal diet containing the essential unsaturated acids. In the muscle where, according to our present belief, a great part of the lipin fatty acid is split off and burnt the turnover of P is one-third as great as in the muscles of the rats receiving the supplements of the essential acids. Since the R.Q. indicates that fat is being burnt and since the basal metabolism is enhanced it is especially interesting to find that more rapid phosphory-

lation and dephosphorylation are taking place in the muscles of the fat-starved rats. This result is in agreement with the view advocated by Sinclair and other workers that a large proportion of the body lecithin is concerned in the metabolism of fatty acids.

Relative rates of turnover in different organs

The ratio of the specific activities of the liver lipin P and muscle lipin P is a rough measure of the rate of lipin P turnover in these organs. A more correct measure would be the comparison of the ratios of the specific activities of the inorganic and lipin P present in the organs. The comparison of the specific activities of the lipin P of various organs does not take into account the different permeabilities of the cells of the various tissues. Thus muscle has been shown to be less permeable to phosphate ions than are the cells of the liver.

Leaving out of account this factor the ratio of the specific activities of the lipin P of muscle, kidney and liver was calculated as 1 : 12 : 21.

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